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ANTIBODY RESPONSE TO NEWCASTLE DISEASE VACCINATION IN A FLOCK OF YOUNG HOUBARA BUSTARDS (*CHLAMYDOTIS UNDULATA*)

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Abstract: Twelve young houbara bustards (*Chlamydotis undulata*) were vaccinated with a lentogenic strain of Newcastle disease virus. Another seven birds were kept in close contact with the treated flock but were not vaccinated. Antibody levels were measured in all birds with hemagglutination inhibition test over the course of 1 yr. Antibody formation with no side effects was observed in 18 birds. The duration and amplitude of the antibody response differed between the groups.

Key words: Chlamydotis undulata, houbara bustard, Newcastle disease virus, vaccination, antibody formation.

BRIEF COMMUNICATION

Newcastle disease (ND) has a worldwide distribution, and velogenic ND virus has been isolated in Saudi Arabia.[4] Birds of all ages are susceptible. Natural or experimental infection has been demonstrated in at least 236 species from 27 orders.[5] Clinical expression of the infection varies among these groups of birds and with the ND virus strain. Juvenile Houbara bustards (*Chlamydotis undulata*) from our captive breeding flock are susceptible to infection with NDV.[8] Because the best protection against ND is through vaccination,[1] our objective was to measure the antibody response following vaccination of young Houbara bustards with a lentogenic strain of ND virus and to ascertain whether unvaccinated individuals in close contact with vaccinated birds developed antibodies to the virus.

This study took place at the National Wildlife Research Center, Taif, Saudi Arabia. At the beginning of the trial, birds were 3 mo old. Five groups of three birds and two groups of two birds were established. Each group was housed in 24-m^2 enclosures with food and water provided ad lib. All birds were accustomed to being handled and examined. They had been vaccinated against fowlpox disease with an attenuated live strain (Diftosec, Rhone Mérieux, Lyon, France) by a wing web technique and were dewormed regularly with 25 mg/kg of fenbendazole (2.5% Panacur, Distrivet, Paris, France). In each group of three and two birds, two and one bird respectively, were randomly chosen to receive an eye instillation of a lentogenic vaccinal strain (Poulvac Hitchner B_1, Solvay Animal Health, Tours, France) at 2.10^6 EID_{50} (mean egg infectious dose) per dose. Twenty-three days later, the same birds received a booster instillation at the same dose. Seven birds of the same age were housed separately, constituting a control group (unexposed controls).

Blood samples were collected from the 12 vaccinated birds (vaccinated group) and the seven nonvaccinated birds in the same enclosures (unvaccinated contacts) prior to vaccine instillation. To test for antibodies, blood samples were collected from all the bustards on days 23 and 30 and every 30 days for 12 mo postvaccination. Blood was collected from the seven control birds following the same protocol. All birds were weighed during blood sampling. Serum samples were evaluated for antibodies by means of a hemagglutination inhibition (HI) test using a beta-micro HI test.[3] Serial twofold dilutions of serum were made in physiological saline containing 8 HA units of ND virus antigen/0.05 ml. The test was performed with a 0.75% suspension of chicken red blood cells at 4°C. HI titers were expressed as the reciprocal of the dilution at which there was complete inhibition of hemagglutination. Data obtained were compared by Mann–Whitney *U*-tests and by Wilcoxon signed ranks tests for paired data. Chosen level of significance was $P < 0.01$.

Twenty-three days after vaccination, the vaccinated birds had at least a $\log_2 7$ increase in antibody titer (from 4 to 512) for four of the birds and a $\log_2 8$ seroconversion (from 4 to 1,024) for eight of the birds. Unvaccinated contacts also showed an increase in antibody titer, from 4 to 512 for two of them and from 4 to 1,024 for four of them. One unvaccinated contact bird presented no antibody increase. The seroconversion was significant ($P < 0.01$) in both flocks. Titers of unexposed control birds remained constant for the next 12 mo (mean (log2) = 2.1; SD = 0.1). A significant difference ($P < 0.01$) in the HI titer was observed between

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Table 1. Newcastle disease virus mean hemagglutinating antibody titers (log_2) over 1 yr following ocular instillation of a live virus vaccine in 3-mo-old houbara bustards.

Birds		Sample day												
			23	30	52	92	122		183	244	275	305	336	362
Vaccinated	12	2.0	9.6[a]	9.8[a]	10[a]	10[a]	9.8[a]	9.5[a]	8.8[a]	7.2[a]	7.1[a]	6.6[a]	6.6[a]	6.5[a]
Unvaccinated contacts	7	2.0	8.8[a]	8.7[a]	8.1[a]	6.7[a]	6.8[a]	5.8	5.8	5.8	5.1	4.7	4.2	4.1
Unexposed controls	7	2.1	2.0	2.2	2.4	2.2	2.1	2.2	2.1	2.2	2.0	2.1	2.1	2.1

[a] Comparisons between vaccinated and unexposed control birds and between unvaccinated contacts and unexposed control birds significant at $P < 0.01$.

the vaccinated group and the unexposed control group after 1 yr postvaccination. Antibody levels were not significantly different ($P = 0.64$) between vaccinated and unvaccinated contact birds until 1 mo postvaccination. In vaccinated birds, a titer >512 persisted for 5 mo and then began to decrease. In the unvaccinated contact group, titers started to decline after 1 mo postvaccination and were not significantly different from unexposed controls at 5 mo postvaccination.

Differences in antibody titer between vaccinated and unvaccinated contacts were significant (Table 1). Vaccinated birds showed greater antibody titer increase. Seroconversion in contact birds was significant despite their not being vaccinated. Spread of vaccinal ND infection among nonvaccinated birds in close contact with vaccinated birds has been described in chickens.[6] A single vaccination with live lentogenic virus in poultry produced a protective titer in susceptible birds of about 16–64.[1] We obtained an HI titer of nearly 1,024 3 wk after vaccination and a titer of 128 6 mo later in vaccinated birds. High persistent antibody level might be indicative of persistent vaccine virus shedding, although the fact that one unvaccinated contact bird never seroconverted and that antibody levels of seroconverted unvaccinated contacts dropped after 1 mo does not support this hypothesis.

Antibodies capable of protecting the host against ND can be measured in virus neutralization (VN) tests. However, because the VN response appears to parallel the HI response, the HI test is frequently used to assess protective response in chickens, especially after vaccination.[2] Although, antibodies directed against either of the functional surface glycopolypeptides, the HN and F polypeptides, can neutralize ND virus,[9] monoclonal antibodies specific for epitopes on the F polypeptides induce greater neutralization than those directed against HN in vitro and in vivo.[7] Therefore, even with seroconversion of up to $log_2 8$, we cannot be certain that a lentogenic vaccine strain of ND virus provides protection against subsequent infection of Houbara bustards with a virulent strain of virus. No clinical side effects were observed. In the present trial, we did not observe differences in the rate of growth between the control and the treatment groups; i.e., vaccination did not appear to influence growth rates in young birds. Use of an avirulent strain of virus as a vaccine can induce postvaccination side effects, with clinical diseases in some species.[1] We did not observe any postvaccination side effects that could have adversely affected the health of these Houbara bustards.

The use of a lentogenic Hitchner B_1 attenuated vaccine at a dosage of 2.10^6 EID_{50} in healthy 3-mo-old Houbara bustards is safe and induced a significant seroconversion 3 wk after vaccination. Following a booster vaccination at the same dose administered 3 wk later, the antibody titer persisted at >64 for 1 yr. Following the same protocol, vaccination of only a part of a flock (12 of 19 birds) induced a similar seroconversion in unvaccinated contact birds. However, these contact birds maintained an antibody titer of >64 for only 4 mo.

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